To the Graduate Council:

I am submitting herewith a thesis written by Eric John Marsland entitled "Tick control and tick transmitted disease monitoring in eastern Tennessee." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Dr. Reid R. Gerhardt, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor
And Dean of the Graduate School

TICK CONTROL AND TICK TRANSMITTED DISEASE MONITORING IN EASTERN TENNESSEE.

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Eric John Marsland
December, 1997

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ABSTRACT

A tick control program targeted at free-living lone star ticks (LST) on white-tailed deer within the community of Fairfield Glade, TN, evolved from an investigation of an outbreak of human ehrlichiosis in that area in 1993. Ivermectin-impregnated corn was fed to deer in an effort to reduce both the reproductive capacity of the lone star ticks and the numbers of free-living individuals from 1994 to 1996. Treated corn was fed to the deer from mid-spring until July 31, 1994 to 1995 and until August 31 in 1996.

Free-living tick stages were sampled in three separate sites in both the untreated and treated areas. One square meter flannel drags were used to sample for adults, nymphs and larval masses. One meter square CO_2 traps were used to sample for questing life stages of the adults and the nymphs.

Reductions were observed among all life stages of LST between 1994 and 1996 in the treated area which is in contrast to the untreated, control area where significant differences were observed among some years, but yearly fluctuations ruled out any pattern. Regression analysis of the number of lone star tick larval masses being produced by female lone star ticks showed ca. 10 times fewer larval masses produced on a per female basis in the treatment area.

Canine populations were evaluated as an indicator species for predicting outbreaks of tick transmitted diseases in human populations. Blood was collected from 53 owner dogs in two communities in Cumberland County and from 104 shelter dogs in Cumberland (n=33) and Knox (n=71) Counties in the spring and fall of 1996 for a series of serological tests to establish exposure to the disease organisms causing canine ehrlichiosis (CE), Rocky Mountain spotted fever (RMSF) and Lyme disease. Evidence of exposure to all three diseases was found in Knox County, whereas exposure to only Lyme disease and RMSF was documented in Cumberland County. Overall, 4, 18, and 14% of dogs tested were positive to the disease organisms causing CE, RMSF, and Lyme disease. Data on canine risk factors were obtained from owner questionnaires and tick collections at owner residences. Initial findings are inconclusive, and additional sampling is necessary to determine the efficacy of using canine populations as a sentinel species for predicting outbreaks of tick-borne diseases.

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CHAPTER I

INTRODUCTION

i - Epidemiology and Public Health Threat of Ehrlichiosis

Tick transmitted diseases such as Lyme disease and Rocky Mountain spotted fever (RMSF) are often a concern for individuals that live and work in tick-infested areas. Recently, a group of disease organisms in the Rickettsia family of bacterial pathogens known as the ehrlichioses have joined the ranks of these public health hazards (Anderson et al. 1992). Human monocytic ehrlichiosis or HME (causative agent *Ehrlichia chaffeensis* Anderson, Dawson, Jones, & Wilson) is currently the most commonly diagnosed ehrlichial pathogen of humans (Gluckman 1997).

Ehrlichia chaffeensis is an obligate, gram-negative, intracellular coccobacillus (Centers for Disease Control 1988). This vertebrate pathogen replicates within the monocytes of the host's immune system eliciting a clinical response in humans that is generally flu-like in nature consisting of a fever, headache, myalgia, and lymphadenopathy (Walker & Dumler 1996). These symptoms vary greatly in their severity and may be misdiagnosed as the influenza. Many antimicrobial therapies which are commonly prescribed for such symptoms are also effective treatments for HME (Gluckman 1997). In Fairfield Glade, TN, 12.5% (N =

311) of the individuals sampled were seropositive for antibodies to *E. chaffeensis* although none of these individuals were clinically diagnosed with the disease (Standaert et al. 1995)

Most recently, a disease known as human granulocytic ehrlichiosis (HGE) has been receiving a great deal of attention. Human granulocytic ehrlichiosis is caused by an *E. equi*-like organism (Centers for Disease Control 1995), but has been implicated as the same species responsible for granulotropic ehrlichiosis of horses and dogs as well (Case 1995). Like HME, HGE is an intracellular parasite, only it affects host granulocytes.

More than 400 cases of HME and 9 fatalities were reported in the United States between 1990 and 1996 with most of these occurring in the southeastern region (Dawson et al. 1996a). Most cases were scattered and sporadic, but two epidemics in Oklahoma and Tennessee indicate the disease can occur in clusters (Fishbein et al. 1991, Standaert et al. 1995). It is important management purposes with all vector-borne diseases to understand the population at risk for.

Prevalence, incidence, and occurrence figures for HGE are not as well known as those for HME (Gluckman 1997). In one study, 29 identified cases were determined from 1994 to mid-1995 in New York state (Centers for Disease Control 1995). This disease is not, however limited to the

northeastern region of the United States with cases occurring in Wisconsin, Minnesota (Bakken et al. 1994), Connecticut, Massachusetts, California, and Florida (Dumler & Bakken 1995).

Those people at risk of contracting HME and HGE are individuals who travel into or live in tick-infested areas. Examples of these include wildlife management areas, parks, and recreation areas, where large numbers of suitable hosts are available to sustain sizeable tick populations. In the southeastern United States, the white-tailed deer (WTD) (*Odocoileus virginianus* Zimmerman) is the supporting host for the lone star tick (LST) (*Amblyomma americanum* L.). The incidence of HME is increasing; presumably due to human encroachment into areas of endemic populations of LST (Standaert et al. 1995).

Although the reservoir hosts and vectors of *E. chaffeensis* are not currently proven, a positive correlation exists between the presence of large numbers of WTD infested with LST, and the presence of antibodies to *E. chaffeensis* within the deer (Lockhart et al. 1995), therefore implicating *A. americanum* in the transmission of *E. chaffeensis*. The bacterium has further been experimentally shown to infect deer (Dawson et al. 1994a) and has been transferred from ticks to both deer (Ewing et al. 1995) and canines(Dawson & Ewing 1992). These experiments indicate both WTD (Dawson et al. 1994b) and domestic canine

populations as potential reservoir or indicator hosts for the disease agent (Dawson et al. 1996[b]).

The vector of HGE, like Lyme disease, is suspected to be *Ixodes scapularis* (Schwartz et al. 1997). Experimental transmission of the causative agent of HGE is possible from *I. scapularis* to uninfected mice (Des Vignes & Fish 1997), but reservoir hosts have not been determined.

Annual incidence of clinical HME is 3-5 cases per 100,000 individuals (Standaert et al. 1995). However, these values could be gross underestimates as many cases may either be sub-clinical or misdiagnosed. People living in communities which encroach into tick-infested areas may be at increased risk of contracting HME. A prime example is Fairfield Glade, Tennessee, a planned resort community built in the forest next to a wildlife management area. In the summer of 1993, an outbreak of HME occurred in Fairfield Glade, TN, in which 11 cases were diagnosed in a community of approximately 4,000 individuals, equivalent to an attack rate of 330 per 100,000 individuals (Standaert et al. 1995).

A Centers for Disease Control (CDC) outbreak investigation team determined the environmental and behavioral risk factors associated with HME exposure. An increased risk of contracting the disease or seroconversion was associated with a history of a tick bite, close proximity to wildlife, retrieving lost golf balls in the rough, high golf score, and failure to use insect

repellants. Conversely, a decreased risk of contracting the disease or seroconversion was associated with use of insect repellants and dropping new balls after hitting into the rough (Standaert et al. 1995).

ii - Tick Control

As a result of the initial outbreak investigation, a tick control program targeting free-living LST on WTD was conducted at Fairfield Glade between 1994 and 1997. Ivermectin-impregnated corn was fed to deer in an effort to reduce both the reproductive capacity of the LST and consequently, the numbers of free-living individuals.

Since the beginning of the project, several insecticides utilizing the feeding delivery method have been proven effective in controlling free living LST. Sonenshine et al. (1996) showed that a treatment method involving permethrin treatment via contact with a modified feeder fitted with a contact delivery device was effective for controlling free-living LST on WTD, and Pound et al. (1996) showed the efficacy of controlling LST in WTD populations living in large paddocks through feeding them ivermectin-impregnated corn. A study design covering 3 years was needed to fully understand the effect of ivermectin on the reproductive viability of adult ticks because LST has a two-year, three-host life cycle.

iii - Serologic Studies

No surveillance system to monitor the disease presence existed in the FFG community prior to the outbreak of HME in 1993. It was suggested that domestic pet populations might be a potential model for predicting future outbreaks (New pers. comm.). Domestic canines were the logical choice as they have been used in the past for such studies (Daniels et al. 1993). The initial stages of a surveillance program require that a baseline be established to determine the "normal" presence of tick transmitted disease organisms within the dog population so that abnormalities can be recognized when they occur.

iv - Research Objectives

The objectives of this research were to determine the effects of feeding ivermectin-treated corn to deer on the reproductive capacity of the LST by completing the research begun by Hutchison (1995). Another aspect was to establish the seroprevalence of antibodies to tick transmitted diseases in domestic canines from two communities in Cumberland County and from two county humane shelters and determine risk factors which would increase or decrease a dog's chance of contracting these diseases to draw parallels to the human population at risk.

CHAPTER II

CONTROL OF FREE-LIVING LONE STAR TICKS BY FEEDING IVERMECTIN-TREATED BAITS TO WHITE-TAILED DEER IN EASTERN TENNESSEE.

i - Abstract

Corn treated at a rate of 50.0 ml of Ivomec® (Ivermectin) pour on insecticide per 22.7 kg of whole kernel cleaned corn was dispensed to deer using four, solar-powered, free-standing deer feeders in 1994, 1995, and 1996. Untreated corn was dispensed for one month at the beginning of every spring, to acclimate the deer to the location and sound of the feeders. Treated corn was placed in the feeders in mid-spring and was dispensed until July 31, 1994 and 1995 and was extended to August 31 for 1996. Free-living tick stages were sampled in three separate sites in both the treated areas and similar untreated areas. Yearly control was achieved within tick life stages by reducing numbers of all life stages, from 1994 to 1996. Reduction in the reproductive viability of females evidenced by fewer larval masses being produced per female found in the treated versus the untreated area was also achieved.

ii - Introduction

Amblyomma americanum (L.), the lone star tick (LST), has been implicated as a possible vector of *Ehrlichia chaffeensis* Anderson, Dawson, Jones & Wilson, the causative organism of human monocytic ehrlichiosis (HME) (Anderson et al. 1992). Currently, HME is one of the three most important tick-related disease problems in the United States (Pound et al. 1996). High populations of LST are associated with large ruminants, particularly domestic cattle and white-tailed deer, *Odocoileus virginianus* (Zimmerman) (Hair & Bowman 1986), and *E. chaffeensis* antibodies in white-tailed deer are strongly correlated with the presence of LST (Lockhart et al. 1996). Human exposure to *E. chaffeensis* and LST is greatest when humans intrude into areas of high deer populations that are supporting large LST populations, which may in turn transmit *E. chaffeensis*. Such a situation occurred during the spring and summer of 1993 at the golfing retirement community of Fairfield Glade, TN, where 11 cases of HME were diagnosed (Standaert et al. 1995). Most of the victims had a history of tick bite and had frequented a newly constructed golf course (Standaert et al. 1995). In this instance, the new golf course was constructed in a forest adjacent to a wildlife management area that was managed primarily for white-tailed deer. Both the management area and adjacent retirement community supported

high populations of white-tailed deer and LST. In addition to the public health threat, the potential economic costs were large because of potential losses in both building and sales of new homes and golf revenues as building lots are priced from $20,000, and annual golf revenues are in excess of 3 million dollars. Fairfield Glade (FFG) is a community that is aggressively marketing both lots and homes and currently has ca. 6,000 residents. The residents and community management were concerned that if transmission of HME continued and FFG obtained the reputation of a tick-infested disease focus, the losses in current property values and lost future revenues could be measured in millions of dollars.

Even though the economic stakes were high, the management of FFG was not prepared to implement the available means of LST control and subsequent HME reduction. The control methods considered and rejected were: 1) area wide acaricidal treatments, 2) deer exclusion, and 3) deer reduction or eradication.

Free-living LST were reduced when resident deer consumed ca. 10 μg ivermectin/0.45 kg of whole kernel corn/day (Pound et al. 1996). At that level of feeding, blood serum levels of ivermectin averaged 21.7 and 28.3 ppb. for the two treatment periods. However, since serum levels of 5-8 ppb. result in almost 100% control of LST (Nolan et al. 1985, Miller et al. 1989), 5 μg ivermectin/ 0.45 kg/day

(50 ml Ivomec®/50 lb. whole kernel corn) was suggested as an appropriate rate (Miller, pers. comm.).

The objective of this study was to determine if reduction of the free-living stages of LST could be achieved by feeding a free-ranging, unrestricted population of white-tailed deer ivermectin treated corn at 5 μg/0.45 kg/day.

iii - Materials and Methods

Fairfield Glade is located in Cumberland Co. on the Cumberland Plateau in eastern Tennessee and has an average elevation of 600 m. This community was begun in 1970 and was built in a second growth mixed mesophilic forest. The overstory consists of mixed hardwoods and pine with sourwood (*Oxydendrum arboreum* L.), white oak (*Quercus alba* L.), white pine (*Pinus strobus* L.), short leaf pine (*P. echinata* Miller), hickory (*Carya* spp.), and black gum (*Nyssa sylvatica* Marsh) predominating. The understory vegetation is variable but consists of blueberry (*Vaccimum* sp.), holly (*Ilex* sp.), wandering jew grass (*Microstegium vimineum*), white pine, white oak, and red maple (*Acer rubrum* L.) seedlings. Road shoulders and other grassy areas (exclusive of fairways and greens) were seeded with fescue and are mowed 3-4 times each year.

The treatment area surrounded a 27 hole golf course and consisted of ca. 450 ha between Catoosa Wildlife Management

Area (WLMA) and Lake Dartmoor (Fig. 2.1). An area of similar size and vegetation south of Lake Dartmoor was chosen as an untreated area. Three tick sampling sites of ca. 10 ha each were selected in both the treatment and non-treatment areas. The sampling sites were subdivided into forest and grassy roadside habitats. Standard LST sampling procedures consisted of two CO_2 traps/plot and 2 flannel tick drags (one each in forest and one on grassy roadside) at biweekly intervals from March to October 1994 to 1996.

Carbon dioxide traps consisted of 1 m square white, slick nylon cloths with ca. 0.5 kg dry ice in the middle (Grothaus et al. 1976). The traps were operated for ca. 1 hr or slightly longer, and the number of adult and larval LST ticks counted and adjusted to 1 hr equivalents. Flannel tick drags were 1 m square white flannel cloths attached to a 2.4 cm diameter wooden dowel that were dragged behind the collector with a rope (Zimmerman et al. 1987). Ten, 10 m drags were made in both forest and grass habitats from March to July. When larval masses were first collected, the number of 10 m drags was increased to 20, but only the first 10 drags were used to estimate adult and nymphal LST, and larval data were adjusted to a 10 drag average. Tick drag and CO_2 trap collections continued until the first frost in October after which no ticks were expected (Gerhardt et al. unpublished data).

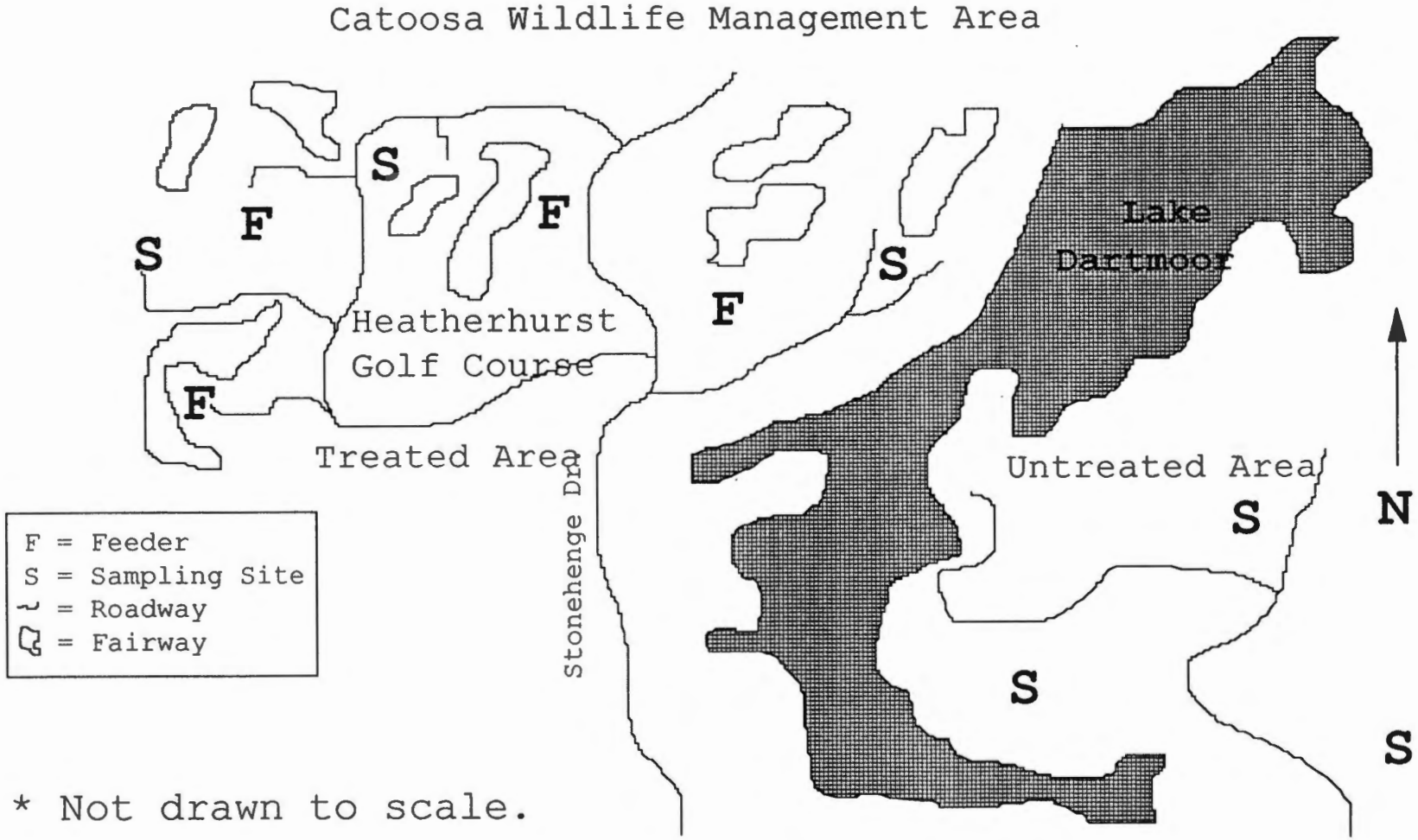

Figure 2.1- Fairfield Glade, TN Tick Study Area, 1994-1996.

Beginning each year in February or March of 1994 to 1996, four, solar powered, electronically-timed, automatic feed dispensers (Specialty Systems, Inc., Austin, TX) were positioned in the treatment area. Feeders were placed in areas of high deer activity as determined by deer signs and sightings, consistent with security and equal distribution within the treatment area (Fig. 2.1). Each year in February or March untreated, whole kernel corn was dispensed twice a day for approximately 4 weeks prior to treatment to accustom the deer to the location, sight, and sound of the feeders. Beginning in March or April, corn treated with 50 ml Ivomec®(5mg/ml Merck pour-on)/22.7 kg was dispensed ca. 1 hr before local sunrise and 1 hr after local sunset. The amount dispensed was adjusted to meet consumption periodically by observing the amount of corn remaining on the ground early in the morning. Dates that treatments began and amounts dispensed are presented in Table 2.1. Treatment ceased on July 31 in 1994 and 1995 to comply with Food and Drug Administration (FDA) requirements to stop feeding 90 days prior to local deer hunting seasons. Feeding continued until August 31, 1996 when the FDA reduced the withdrawal time to 60 days prior to deer season (George, pers. comm.).

The corn was treated with ivermectin by placing 22.7 kg in an electric concrete mixer with 50 ml Ivomec® pour-on for 3 min (Pound et al. 1996). Treated corn was repackaged in

Table 2.1. Dates of start and finish and amounts of corn dispensed for pretreatment conditioning period and treatment period, Fairfield Glade, TN, 1994-1996.

	1994	1995	1996
Pretreatment			
Start Date	3-11	2-6	2-17
Finish Date	4-8	3-13	3-22
Amount (kg)	544.8	544.8	725.7
kg/day	19.5	15.6	21.3
Treatment			
Start Date	4-8	3-13	3-22
Finish Date	7-31	7-31	8-31
Amount (kg)	1611.7	2179.2	241
kg/day	14.1	15.6	14.9

the original bags and then placed into the feeders as they became depleted. The feeders were reliable and only in a few instances were there interruptions in the regimen. In 1995 and 1996, the feeders were modified by enclosing the dispensing section of the feeder with 2.5 cm (1 in.) chicken wire to prevent squirrels from extracting corn. This modification only slightly reduced the distance corn was dispersed from the base of the feeder. Additionally, sunflower seeds were made available near the feeders ca. 3-4m above the ground in 1996 to distract squirrels from the corn.

No estimates were made of deer populations because of time constraints and the variable nature of the deer herds in this area. Deer were, however, common. Fresh feeding signs, tracks, and scats were observed at all collection sites and feeder locations. We continually observed adult deer and fawns from the road and while servicing the feeders and making tick collections. Deer are considered a pest of home ornamental plantings by many residents although in another study, ca. 31% of residents regularly presented food for deer or other animals. In this same study concerning the prevalence of antibodies to tick transmitted disease organisms in canines ca. 46% resident dog owners questioned regularly saw deer or had deer feeding in their yard (see CHAPTER III).

Data were analyzed in SAS using several procedures. Since the data were not normal, they were transformed using a $\log_{10}$ transformation, however data are presented in their untransformed state. Normality of the data was checked using PROC UNIVARIATE (SAS 1989). The linear model (PROC GLM (SAS 1989)) used was $y_{ijklm}= a_i+b_j+c_k+d_l+e_{ijklm}$, where a=year, b=site, c=habitat, d=sampling method, y_{ijklm}= tick life stage, and e is the residual error. Mean separation was performed using Duncan's Mean Separation at $\alpha=0.05$. Yearly control values and percent reductions were calculated using Abbott's Formula (Abbott 1925).

The yearly life-stage averages by site were used in a linear regression model (PROC REG [SAS 1989]) relating females to the larval masses they produce. In both cases, the models used were statistically significant; $p<0.01$ and $p<0.05$ for the treated and untreated sites respectively. Larval mass production was related to the number of females found in both the treated and untreated areas. Sixty-seven percent and 52% of the larval mass production differences could be explained by differences in the number of females found in the treated and untreated areas, respectively.

iv - Results and Discussion

Feed Consumption

The inclusive dates and amounts of corn dispensed for pretreatment feeding and ivermectin treatment feeding for 1994-96 are presented in Table 2.1. Consumption in the conditioning period started low and increased as the deer discovered and became accustomed to the feeders. The feeders remained in the same place each year, and conditioning consumption increased. No noticeable reduction in consumption was observed when treated corn was presented. A noticeable reduction in consumption, however, occurred in April when the deciduous trees and understory vegetation began to leaf out and compete with the corn for consumption. The leafing out coincided with fawning which further reduced utilization of the corn by females with fawns who are generally more reluctant at that time to travel far for forage. As the summer progressed, corn consumption returned to pre-leafing levels. Only a few instances of feeder failure occurred in which the batteries either failed or became disconnected, and in one instance a feeder was stolen. It was replaced within 3 weeks.

Tick Collections

The collections of free-living female, male, and nymphal LSTs indicate that while we tried to select an

equivalent untreated area on the basis of topography and vegetation, initially considerably more LST were in the treatment area than in the untreated area (Fig. 2.2A & B). While we would have preferred to have had an untreated area with similar LST populations, none was available that met our need of proximity, security, and access. The seasonal occurrence of LST in the untreated area was similar to that reported for other states except that adult activity started later in the spring followed by a corresponding delay until late August before the larvae appeared in large numbers (Gerhardt et al., in press). Subsequent collections (1995 and 1996) showed reduced percentages of females (61%), males (66%), and nymphs (80%) when compared to 1994 levels. Although 1994 was a treatment year, the free-living adults and nymphs were the result of successful attachment of nymphs and larvae in 1993 and consequently are to be considered as pretreatment population estimates. Larval masses in the treatment area were not reduced to the same extent as the adults and nymphs (43%) which was due to the fact that the larvae in 1994 were the first free-living stage sampled to have been influenced by the ivermectin treatment.

The yearly averages by site were used in a linear regression model plotting females by the larval masses they produce. Figure 2.3 shows the calculated lines passing through the data points indicating the average numbers of

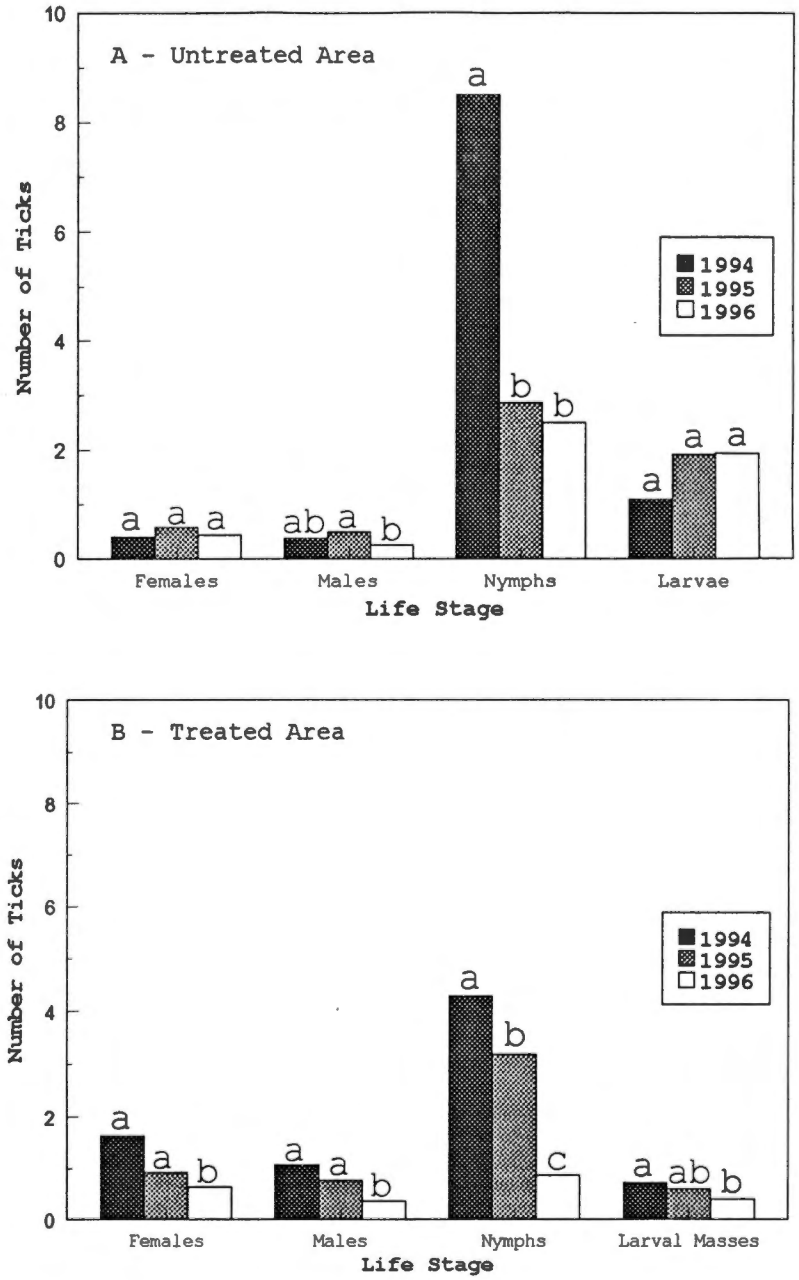

Figure 2.2- Average number of lone star tick life stages found per collection date grouped by life stage in treated and untreated areas of Fairfield Glade, TN, 1994-1996.

Bars with the same letter are not significantly ($p>0.05$) different; bars represent non-transformed data, however statistical analysis was performed on transformed data.

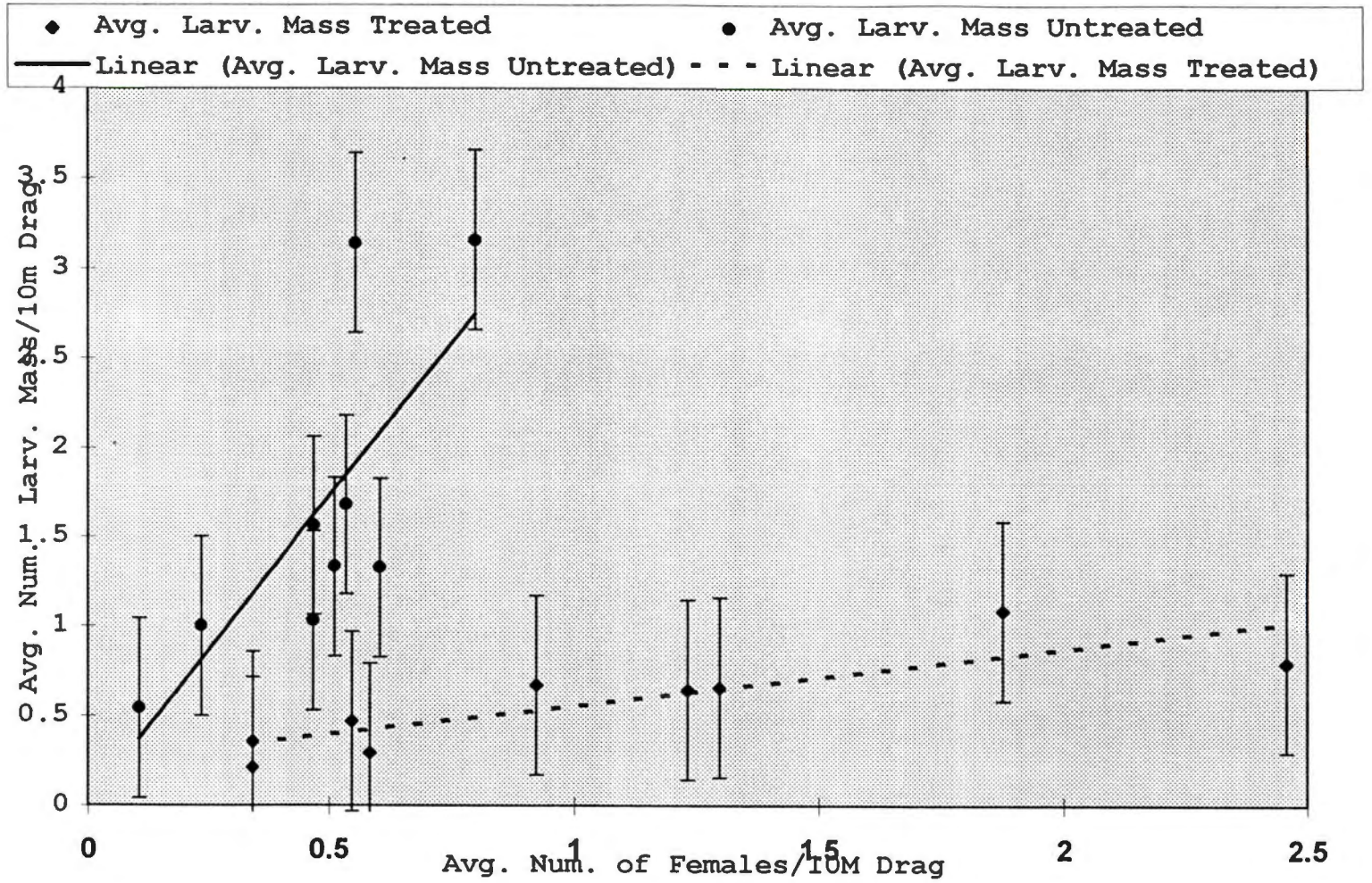

Figure 2.3- Average Female to Larval Mass Transition: Regression Analysis for Fairfield Glade, TN, 1994-1996.

females and larval masses found in a collection area per day. On a per female basis, more larval masses were produced in the untreated area than in the treated area.

With the exception of females, the percent reduction for each stage was greater from 1995 to 1996 than 1994 to 1995 (Table 2.2) which may have resulted from increased ivermectin-treated corn consumption (Table 2.1) and the provision of sunflower seeds to reduce squirrel consumption of corn.

Treatment Effect

In each year, the numbers of larval masses in the untreated area were greater than those collected in the treatment area which was true even though significantly more adults were in the treatment area initially. Nymphal data was erratic. Two, 10 m drags in 1995 yielded 128 and 89 nymphs in the treated area, thus driving the percent reduction down. Fewer larval masses were produced from year to year in the treatment area when compared to the untreated area, and there were 6 to 7 times more larval masses were produced per adult female in the untreated area than in the treated area (Fig. 2.3). Successive reductions (some significant) in all life stages among years were also observed in the treatment area but not in the untreated area. These data clearly show the adverse effect of ivermectin treatment on the combination of adult survival

Table 2.2. Average numbers of ticks found per collection date in the treated and untreated regions of Fairfield Glade, TN, and the percent reductions found in the treated area among life stages, 1994, 1996.

	Fem[1]	Fem	Mal[2]	Mal	Nym[3]	Nym	Larv[4]	Larv
TRT	**Avg**	**%red**	**Avg**	**%red**	**Avg**	**%red**	**Avg**	**%red**
1994[5]	1.63	N/A	1.07	N/A	4.28	N/A	0.72	N/A
1995	0.92	44	0.78	27	3.19	26	0.60	16
1996	0.63	31	0.36	55	0.87	73	0.40	33
Tot[6]		61		66		80		44
	Fem	**Fem**	**Mal**	**Mal**	**Nym**	**Nym**	**Larv**	**Larv**
UNT	**Avg**	**%red**	**Avg**	**%red**	**Avg**	**%red**	**Avg**	**%red**
1994	0.40	N/A	0.38	N/A	8.51	N/A	1.08	N/A
1995	0.57	42	0.49	-30	2.86	66	1.91	-77
1996	0.44	-24	0.25	50	2.49	13	1.94	-1
Tot		-8		35		70		-79

[1] Fem = Female
[2] Mal = Male
[3] Nym = Nymph
[4] Larv = Larval Masses
[5] Yearly % reductions are calculated by Abbott's Formula from current year to previous year. No data were available for 1993, so 1994 reductions are not applicable.
[6] Tot. Reflects the total reduction from 1994 to 1996.

and/or fecundity of LST adults. The fact that the adult populations were not reduced to the extent observed by Pound et al. (1996) is almost certainly due to the free movement of deer into and out of the treatment area thus replenishing LST of all stages.

This study shows that while complete elimination of LST in an extended area is improbable, considerable overall reduction of adults and larvae is possible with extended effort. These reductions may well be all that is required since reduction or elimination of disease organism transmission does not require tick eradication. A coupled effort to educate residents about ways of reducing their risk of coming in contact with the ticks and a tick control program could well be sufficient to reduce human risks to negligible levels.

CHAPTER III

SEROPREVALENCE OF ANTIBODIES TO SELECTED TICK TRANSMITTED DISEASE ORGANISMS IN EASTERN TENNESSEE.

i - Abstract

Blood was collected from 53 owned dogs in two communities in Cumberland County, TN, and from 104 shelter dogs in Cumberland and Knox Counties, TN. Serological techniques (IFA, and ELISA) were used to determine the exposure of the dogs to the disease organisms causing Rocky Mountain spotted fever, Lyme disease, and canine ehrlichiosis. Data on canine risk factors for exposure to these disease organisms were obtained from owner questionnaires and by tick collections at owner residences. The presence of white tailed deer and in turn the lone star tick appear to be factors influencing the canine exposure status for the owned dog population. A polymerase chain reaction technique testing for both *E. chaffeensis*, and *E. phagocytophila* is currently being established.

ii - Introduction

Human monocytic ehrlichiosis (HME) was once considered to be caused by the same agent of canine ehrlichiosis (CE) (*Ehrlichia canis* Donatien and Lestoquard) (Maeda et al. 1987), but has since been determined to be *E. chaffeensis* (Dawson et al. 1991a). Recently, a number of new ehrlichial diseases have emerged with the advent of more sophisticated diagnostic tests and molecular techniques (Walker & Dumler 1994).

In the summer of 1993, an outbreak of HME in the retirement golf community of Fairfield Glade, TN, prompted the Centers for Disease Control to send an outbreak investigation team to study the area. Standaert et al. (1995) established a number of risk factors which would increase an individual's chances of contracting the disease. If similar or equivalent risk factors could be established for the canine residents, a surveillance system might be established to warn of future outbreaks.

Animals have long been used as biological indicators for environmental hazards such as the proverbial "canary in a coal mine". They have been used in several instances to determine environmental risks for humans. Domestic dogs have been used as epidemiological models for predicting the occurrence of human diseases such as various types of environmentally induced cancer as their exposure to certain

disease factors should be similar to those experienced by their owners (Garbe 1988).

Dogs seroconvert to many of the same tick-vectored disease organisms as humans, and thus, may serve as biological predictors for potential outbreaks of these diseases for human populations (Daniels et al. 1993). Canines seroconvert and develop clinical infections to the agents of Lyme disease, ehrlichiosis (HME and CE), and Rocky Mountain spotted fever (RMSF) (Reinemeyer & Patton 1995). Dogs and deer have been used to examine the seroprevalence of the causative agent for Lyme disease (*Borrelia burgdorferi*) and HME (Daniels et al. 1993, Dawson et al. 1996a). The prevalence of the Lyme disease vector on and off of white-tailed deer (WTD) and the seroprevalence of antibodies to the disease organism in both WTD and canine populations were also determined (Daniels et al. 1993). Dawson et al. (1996 a) worked with a population of shelter dogs with little information available on their health and behavioral histories.

Effective sentinel models should have the essential information necessary to allow proper inferences and parallels to be made to human populations. Exposure to tick-borne disease organisms is different for a seasoned hunting dog and an indoor, toy poodle. Thus, behavioral, environmental, and medical history information must be collected to help make these connections to the human

population at risk. An effective canine sentinel model would show evidence of exposure before the disease becomes a risk for the human population.

Testing for the occurrence of tick-transmitted disease organisms has become easier with the advent of diagnostic procedures such as the indirect fluorescent antibody test (IFA), enzyme-linked immunosorbent assay (ELISA), and the polymerase chain reaction (PCR). Canine ehrlichiosis, HME, and RMSF antibodies can be detected by IFA's. IFA tests are good indicators of a past exposure caused by a *Ehrlichia spp.* For treatment purposes, a period of several weeks can pass before a patient would test positive by such tests, so it would be beneficial to have those results right away. Molecular techniques, such as testing for the presence of *Ehrlichia spp.* by a nested PCR, are quickly becoming the tests of choice by clinicians as they can determine if the disease agent is present almost immediately (Gluckman 1997). The RMSF IFA is currently the diagnostic test being used by the University of Tennessee College of Veterinary Medicine to differentiate convalescent from non-convalescent dogs (Doan pers. comm.).

PCR has recently been used as a definitive diagnosis for the presence of *Ehrlichia spp.* The PCR test used was developed as a nested reaction first amplifying a broad range of DNA from bacterial pathogens followed by a secondary, species amplification step with a final positive

demonstrated through agarose gel electrophoresis with ethidium bromide visualization (Dawson et al. 1996b).

iii - Materials and Methods

Paired Community Selection and Owner Questionnaire

Two residential communities, Fairfield Glade (treatment) and Lake Tansi (unaffected control), were selected in Cumberland County, TN as sample sites for collections of samples from owned dogs. These two communities were both the "golf-oriented" communities used by Standaert et al. (1995) in his investigation of the HME outbreak in 1993, and were selected because their boundaries are separated by greater than 30 km. Resident dogs should, therefore, belong to isolated and independent populations.

Sample Collection

Blood was collected from resident dogs in both FFG and LT during planned and previously publicized clinics open to the residents in the spring and fall of 1996. The FFG clinics occurred on March 29 and December 20 of 1996. The LT clinics were on April 20 and again on December 20 of 1996. Owners were asked to fill out a questionnaire concerning information on the dog's medical history and habits (Appendix I) to identify factors influencing the dog's susceptibility to exposure, and in turn, infection or

seroconversion. This questionnaire was based on the one used by Standaert (1995) for the human residents of these communities. Owners of dogs which returned for the second clinic were asked only for information which might have changed the dog's exposure or disease status since the last clinic. Owners also were asked for permission to visit the home site.

Home Site Visits

Home site collections were made in June, 1996 and 1997 following the spring and fall blood collections, respectively, to determine on-site tick burden. An attempt was made at each home site to determine what kind of environment the dogs lived in, and through a dragging sampling method, if and what species of ticks were present. A rating sheet was used (Appendix II) as a template for data collection for information on the dog's environment. Rating scales were used to determine what types of vegetation and percent cover (overstory and understory) were present at the home site and on adjacent lots.

Home site tick collections were made using 1.0 m^2 white flannel drags (Zimmerman et al. 1987). Ten, 10m drags were done in each yard. Ideally, 3 drags were done in both the front and back yard with 2 drags on both the left and right side yards. These were adjusted proportionally if the yard layout and vegetation did not allow. Collections on

adjacent lots (front, back, and sides) were conducted in a similar fashion, in that ten, 10m drags were performed exactly as described above if a home was present and permission was granted by the homeowner. If an undeveloped or open adjacent lot was present, 10, 10m drags were performed randomly throughout the lot. Ticks found at the homesite along with ticks on the adjacent sites were kept and identified.

Animal Shelter Sampling

Blood was collected from dogs at the animal shelter operated by the Humane Shelter of the Tennessee Valley (HSTV) in Knox County, TN (N=71), and the Cumberland County Humane Society animal Shelter (CCHS) in Crossville, TN (N=33), in 1996. Collections were made in Knox County on a biweekly basis from May 1996 to September 1996. Collections at the Crossville shelter were done on August 15, 1996 and again on December 20, 1996. The dogs were assigned a reference number, and sex and approximate age were noted at that time.

Blood Collections and Storage

Blood was collected from the foreleg with 3 cc syringes and was expelled into 3 cc vacutainers containing EDTA (an anti-clotting factor). Blood was stored in a cooler with ice for the trip back to the laboratory at which point the

blood was fractioned into whole blood and plasma aliquots.

Whole blood aliquots were needed for the PCR test, so an aliquot was taken from each tube and was stored at -20°C. Plasma needed for the antibody type tests was collected from the remaining whole blood by centrifugation for 5 min at 3500 rpm in a Dynac II Centrifuge fitted with a swinging rotor (Clay Adams Mfg.). If the plasma tests were to be run within two weeks of the collection date, they were not frozen, but were maintained at 4°C until they were tested and were then subsequently stored at -20°C.

Serological Testing

A series of three primary-type antibody assays testing for the presence of RMSF, *E. canis*, and Lyme disease was used. A 1:40 and a 1:80 serial dilution of plasma was made for each sample. Samples were diluted with 1X phosphate buffered saline (PBS). The IFAs for both *E. canis* and RMSF were prepared as described previously (Dawson et al. 1991b), and were examined for fluorescence with a Zeiss ultra-violet microscope. Successive, serial dilutions were not performed on sera testing positive to *E. canis,* but if the dog tested positive at a dilution of 1:80 for RMSF, successive serial dilutions were made to 1:160, and 1:320.

The *E. canis* antigen slides for the IFA were purchased from the Veterinary Medical Research and Diagnostic (VMRD) Corporation, and the RMSF test was produced at the

University of Tennessee College of Veterinary Medicine (UTCVM) with antigen which was purchased from a commercial outfit.

The test for Lyme disease exposure was the commercial ELISA, LymeCHEK®, developed by the Synbiotics Corporation. The protocol accompanying the test kit was used as directed for testing whole, undiluted plasma using the reagents provided. A positive test was indicated with a change in color or a shade of blue in this case (Synbiotics Corp. San Diego, CA, 1994).

Once the results were found for the sero-tests, a letter was sent to the dog owners indicating the results and ramifications of the tests. In that letter (Appendix 3), an offer was made to the owners to have a re-test performed on their dog's blood at no charge if they had it submitted by their veterinarian.

DNA Extraction

DNA was extracted from the whole blood specimens using a United Stated Biochemical Corporation DNA isolation kit (catalog #73750) designed specifically for whole blood samples following the procedure outlined in their fast method (USB Cleveland, OH, 1997). The final pellet was resuspended in 100μl of RNAse-free H_2O.

DNA Amplification and Agarose Gel Analysis

A nested PCR was attempted on the extracted DNA for the presence of *Ehrlichia*-like DNA. The outside amplification was attempted using 5μl of extracted template DNA in a 50μl reaction mixture containing 10mM Tris-Cl (pH 8.3), 50mM KCl, 1.5mM $MgCl_2$, 0.2mM each dNTp, 5μM tetramethylammonium-chloride (TMAC), 1.25 units Ampli*Taq* Gold® polymerase (Perkin-Elmer/ Applied Biosystems Division [PE/ABD] Foster City, CA), and 0.8 μm of 8F (5′-AGTTTGATCATGGCTAG-3′) and 1448R (5′-CCATGGCGTGACGGGCAGTGTG-3′). An initial incubation of the reaction mixture for 10 min at 95°C was needed to activate the enzyme as per the manufacturer's instructions. This reaction mixture was then amplified in a thermocycler (MJ Research, DNA Engine 2) for 40 cycles: 1 min at 94°C, 45°C for 2 min, and 72°C for 2 min and was followed by a 7 min 72°C annealing step and was held at 4°C overnight.

The nested PCR was conducted using the same reaction mixture described above with the exception of switching out primers, using 5μl of the amplification product from the first reaction, and altering the amplification times and temperatures. *E. chaffeensis* DNA amplification was attempted using HE1 (5′-CAATTGCTTATAACCTTTTGGTTATAAAT-3′) as the forward primer paired with HE32 (5′-TATAGGTACCGTCATT-ATCTTCCCTAT-3′) as the reverse primer. Human granulocytic ehrlichiosis amplification was attempted using GE9F (5′-AACGGATTATTCTTTATAGCTTGCT-3′) as the forward primer paired

with GE10R (5′-TTCCGTTAAGAAGGATTCAATCTCC-3′) as the reverse primer. Again, a 10 min incubation at 95°C was necessary to activate the enzyme, and the temperature profile used was 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min for 40 cycles. PCR products were run on a 1.2% agarose gel containing ethidium bromide, and were visualized under UV illumination (Dawson, Pers. Comm.). A photograph was taken for a permanent record.

Statistical Analysis

Data were analyzed using the Epi Info software package. Pairwise comparisons and T-tests were done relating exposure status to behavioral and health characteristics. The 95 percent confidence intervals for relative risks were performed in the CSAMPLE questionnaire data management section of the program (Centers for Disease Control 1997).

iv - Results and Discussion

Antibodies to all of the tick-transmitted disease organisms being tested for were present in dogs from Knox County. Cumberland County did not have any CE positive dogs, but had dogs positive for RMSF and Lyme disease. There were 159 dogs surveyed in Knox and Cumberland Counties of which 6, 28, and 23 tested positive for CE, RMSF, and Lyme disease, respectively (Table 3.1).

Table 3.1. Antibody prevalence for RMSF[1], CE[2], and Lyme disease in canine blood for samples collected in 1996 in Cumberland and Knox Counties, TN.

Location	Date	N*	CE	%**	RMSF	%	Lyme[3]	%
FFG[4]	3/29	31	0	0	3	10	12	39
	12/20	15	0	0	5	33	0	0
FFG-Tot.		**46**	**0**	**0**	**8**	**17**	**12**	**26**
LT[5]	4/20	7	0	0	0	0	1	14
	12/20	2	0	0	0	0	0	0
LT-Tot.		**9**	**0**	**0**	**0**	**0**	**1**	**11**
CCHS[6]	8/15	16	0	0	6	38	2	13
	12/20	17	0	0	3	18	0	0
CCHS-Tot.		**33**	**0**	**0**	**9**	**27**	**2**	**6**
HSTV[7]	1996	71	6	8	11	15	8	11
TOTAL	**1996**	**159**	**6**	**4**	**28**	**18**	**23**	**14**

[1] RMSF= Rocky Mountain spotted fever positive titered at 1:40
[2] CE= canine ehrlichiosis positive titered at 1:40
[3] Lyme= Lyme disease positive tested on whole plasma
[4] FFG= Fairfield Glade, TN
[5] LT= Lake Tansi, TN
[6] CCHS= Cumberland County Humane Shelter
[7] HSTV= Humane Society of the Tennessee Valley
* N= number of canines sampled by collection date
** %= percentage of positive tests by disease type

Rocky Mountain Spotted Fever

Evidence of exposure to Rocky Mountain spotted fever was found in both Knox and Cumberland Counties. In FFG alone, 17% of the dogs surveyed had a positive titer to RMSF. The CCHS had similar results with 27% of 33 dogs surveyed positive for RMSF antibodies. This indicates a potential disease focus of RMSF in Cumberland County. However, 0% of the 9 dogs surveyed in LT were positive for RMSF titers. This could be a result of the relative absence of ticks in the LT region as was evidenced by collection data at home sites.

The HSTV in Knox County had a total of 11 RMSF or 13.6% positives from 71 dogs surveyed which is not much different from 18% especially for a small sample. Knox County therefore, also may have a RMSF disease focus.

Lyme Disease

Lyme disease antibodies also were found in both Knox and Cumberland County dogs. However, bias due to vaccination, and unreliable tests cast doubt on the validity of these test results. Little information can be extrapolated from this data.

In FFG, 14 of the 43 owners (32.6%) who answered the question had a vaccination administered to their dogs against Lyme disease. Of the 12 dogs who tested positive for Lyme disease, 7 had been reported vaccinated and 5 had

not according to their owners. Dogs whose owners said they had been vaccinated are 3.73 (1.45,9.64) times more likely of having antibodies to Lyme disease, thus testing positive to the ELISA based LymeCHEK® test. False positive tests for the Lyme ELISA might also be the result of a residual titer due to a vaccination against the causative agent of Lyme disease, *B. burgdorferi*. Commercial vaccines are available and are being administered by veterinarians (Fort Dodge Animal Health).

A majority of these dogs in FFG (29 of 44- 66%) had also spent a significant amount of time away from Cumberland County at some point. Eight of the 12 *B. burgdorferi* positives had, according to their owners, traveled away from their residence (4 of the 12 positives in Michigan alone). The majority of the human cases of Lyme disease occur in the northern portions of the United States (Centers for Disease Control 1989) suggesting these data might not be a reflection of seroprevalence levels in TN but seroprevalence values of other regions in the country.

Eight of the 71 dogs tested at the HSTV were positive for antibodies to Lyme disease. Since little is known on these dogs, these data need to be examined closely.

Canine Ehrlichiosis

All 6 *E. canis* positives came from the Humane Society of the Tennessee Valley Shelter in Knox County. Further

testing will be necessary to determine its true presence in eastern Tennessee.

Owner Questionnaires

Fairfield Glade had a total of 44 owners complete the questionnaires, and Lake Tansi had a total of 6 owners completing the questionnaires. Results are displayed in Tables 3.2 and 3.3 for FFG and LT, respectively.

A total of 15 dogs tested positive to at least one of the tests in FFG representing 8 RMSF and 12 Lyme positives. Of these test positive dogs and the 13 owners who answered the question, 8 were considered to be indoor dogs and 5 were considered indoor/outdoor which is not unlike the results found for all of FFG where 29 owners indicated 2 dogs as outdoor, 15 as indoor, and 12 as indoor/outdoor.

Owner questionnaire data show the majority of the owners taking interest in the care of their animals. Seventy-four percent of the FFG residents surveyed administer heartworm preventives. Of the FFG residents whose dogs tested positive for any of the three disease tests, 12 received and 3 did not receive heartworm preventive as opposed to 20 and 9, respectively, for those not testing positive to any of the tests.

The presence of deer, and in turn, ticks in Cumberland County differs between FFG and LT. Owners at both locations commonly reported deer feeding on their property. Forty-

Table 3.2. Owner questionnaire summary for Fairfield Glade, TN: spring and winter collections combined, 1996.

Question	n[1]	N[2]	%[3]
Residents who leave food/salt for deer	5	44	11.4
Residents who leave food for animals	14	44	31.8
Residents who see deer eating in yard	20	44	45.5
Dogs which left the residence/extended	29	44	65.9
Type of dog- Outdoor	2	43	4.7
Indoor	24	43	55.8
Indoor/Outdoor	17	43	39.5
When the dog is outside- Leash	27	43	62.8
Free- Running	11	43	25.6
Fenced In	5	43	11.6
Owners That Find Ticks	38	44	86.4
Frequency of Tick Finding-Frequently	8	38	21.1
Occasionally	18	38	47.4
Infrequently	4	38	10.5
Rarely	8	38	21.1
Dogs that Wear a Flea/Tick Collar	26	41	59.9
Owners that Use Flea/Tick Treatments	34	44	77.3
Dogs Vaccinated Against Lyme Disease	14	43	32.6
Dogs on Heartworm Preventatives	32	43	74.4

[1] n = Number of those owners answering yes.
[2] N = Total number of owners surveyed.
[3] % = n/N X100

Table 3.3. Owner questionnaire summary for Lake Tansi, TN: spring and winter collections combined, 1996.

Question	n[1]	N[2]	%[3]
Residents who leave food/salt for deer	0	6	0.0
Residents who leave food for animals	1	6	16.7
Residents who see deer eating in yard	1	6	16.7
Dogs which left the residence/extended	2	6	33.3
Type of dog- Outdoor	1	6	16.7
Indoor	4	6	66.7
Indoor/Outdoor	1	6	16.7
When the dog is outside- Leash	4	6	80.0
Free- Running	1	6	20.0
Fenced In	0	6	0.0
Owners That Find Ticks	5	6	83.3
Frequency of Tick Finding-Frequently	0	5	0.0
Occasionally	3	5	60.0
Infrequently	0	5	0.0
Rarely	2	5	40.0
Dogs that Wear a Flea/Tick Collar	2	6	33.3
Owners that Use Flea/Tick Treatments	6	6	100.0
Dogs Vaccinated Against Lyme Disease	1	6	16.7
Dogs on Heartworm Preventatives	5	6	83.3

[1] n = Number of those owners answering yes.
[2] N = Total number of owners surveyed.
[3] % = n/N X100

five percent of dog owners in FFG and 16.7% of LT dog owners observe deer eating on their property regularly. Of the 15 seropositive dogs in FFG, 12 of the 15 owners had found ticks on their pet. One had found them frequently, 9 had found them occasionally, and 2 had found them rarely. Of the negative FFG canines answering the question (N=29), 26 had found ticks on their pet. Seven of the negatively tested FFG canine owners indicated finding ticks frequently, 9 occasionally, 4 infrequently, and 6 rarely.

In Lake Tansi, of the 7 owners who participated in the survey, 5 indicated that they find ticks on their pet. Of those 5, 3 indicated finding them occasionally, and 2 indicated finding them rarely. Two reported never finding them at all. The presence of WTD and in turn LST in FFG may be a substantial risk factor for RMSF and Lyme disease.

Return Dogs

Even though the study was designed to collect duplicate samples from owned dogs, only 4 owned dogs returned for a collection of a second blood sample. Percentages in the text and tables reflect the values of these dogs only once.

Home Site Visits

Both in FFG and LT, the majority of the dog-owners lived in single family homes. Habitat notations were the same for the two communities in that both the overstory and

the understory plant species present were nearly identical being a mixed hardwood overstory and a brushy mixed blueberry understory. FFG and LT had an equal proportion of developed and undeveloped lots near the homesite.

Site visits confirmed what the dog owners had indicated on their questionnaires. Fifty percent of residence surveys (adjacent and homesite collections combined) were positive for the presence of LST whereas only 11% of the LT home sites (adjacent and homesite collections combined) were positive for ticks. The ticks found at the homesite in LT were all American dog tick, *Dermacentor variabilis*. Details regarding on-site and adjacent-site tick collections are found in Table 3.4.

Fairfield Glade has a much higher deer population than LT, and in turn a higher LST population. The high deer population may reflect FFG's close proximity to Catoosa Wildlife Refuge, which shares an adjacent border. The mere presence of deer (governed by location), and in turn, the LST, may constitute the greatest risk factor dogs experience in tick associated disease transmission in Cumberland County. Additional surveys and diagnostic tests, such as the PCR, will be necessary for any long term project to determine the actual risks of exposure in eastern Tennessee.

Table 3.4. Results from the June 1995 and 1996 site visits from Fairfield Glade and Lake Tansi, TN.

Date/Loc.	HS[1]+	HS-	N	AD[2]+	AD-	N	% of pos.[3]
June 1996/FFG[4]	11	15	26	22	17	39	51%
June 1997/FFG	5	4	9	7	9	16	48%
Total FFG	16	19	35	29	26	54	50%
June 1996/LT[5]	1*	5	6	1*	8	9	13%
June 1997/LT	0	1	1	0	3	3	0%
Total LT	1	6	7	1	11	12	11%

[1] HS = Home Site Visits
[2] AD = Adjacent Site Visits
[3] % of pos. = Percentage of Home Site and Adjacent Site Positives.
[4] FFG = Fairfield Glade, TN
[5] LT = Lake Tansi
* + and - indicate ticks and no ticks, respectively.
** All LT positives were for *Dermacentor variabilis*, whereas all FFG positives were *Amblyomma americanum*.

Relative Risk Analysis

The relative risks were calculated using a pairwise comparison format looking for correlations among exposure or test status and behavioral and health history information. This analysis indicated little significance in the relationships between living in FFG versus living in LT, comprehensive behavioral and health history risk factors, and risk factors associated with living in Knox versus Cumberland Counties. Data for these along with 95% confidence intervals can be found in Tables 3.5, 3.6, and 3.7. With the exception of the association between dogs which had been vaccinated against Lyme disease and dogs which tested positive to the ELISA for Lyme disease, no significant risks were found in any of the comparisons. A larger sample size would help elucidate the true relative risks a dog would be subjected to in eastern Tennessee.

Polymerase Chain Reaction

The initial two runs of the PCR diagnostic testing for *E. chaffeensis* and the causative agent of HGE were unsuccessful. Amplification was not achieved for either the positive control or any of the blood samples. The protocol is being examined to see where errors might have occured.

Table 3.5. Relative risks and 95 percent confidence intervals calculated for determining differences in environmental risk factors between Fairfield Glade and Lake Tansi.

Characteristic	FFG Owners Evidence of Characteristic no./total no. (%)	RR[2] (95% CI[3])
Lyme ELISA	+:12/13[1] (92.3) -:34 /42 (81.0)	2.35[4] (0.34,16.15)
RMSF IFA	+:8/8 (100) -:38/47 (81.0)	----------
Deer seen in yard	Y:20/21 (95.2) N:24/30 (80.0)	3.18 (0.50,20.44)
Find ticks	Y:38/44 (86.1) N:6/7 (85.7)	1.00 (0.73,1.40)
Dogs which left residence/extended	Y:29/31 (93.5) N:15/20 (75.0)	2.31 (0.69,7.67)
Flea/Tick collar	Y:26/28 (92.9) N:18/23 (78.3)	2.07 (0.62,6.92)
Other flea/tick treatments	Y:34/41 (82.9) N:10/10 (100)	0.773 (0.66,0.91)
Lyme vaccine	Y:14/15 (93.3) N:29/35 (82.9)	2.28 (0.35,14.97)
Heartworm preventive	Y:32/38 (84.2) N:11/12 (91.7)	0.87 (0.61,1.24)
Ticks Found on Site Visit	Y:17/18 (94.4) N:20/26 (76.9)	3.22 (0.50,20.76)

[1] Results are read "Out of the 13 dogs testing positive for Lyme disease, 12 were from FFG.
[2] RR= Relative Risk
[3] CI= Confidence Interval
[4] Additional risk over and above that experienced by animals not exposed to the characteristic.
* Since all confidence intervals include (1), none are significant($\alpha = 0.05$).
** Numerators not adding up to 46 indicate missing values.
*** Denominators not adding up to 55 indicate missing values from the owner questionnaire.

Table 3.6. Relative risks and 95 percent confidence intervals used for determining health history, behavioral, and environmental risk factors for owned dogs in Cumberland County, TN.

Primary Category		Secondary Category		Percent[1] no./Tot no.	RR[2] (95% CI[3])
Lyme Dis.	+	Lyme	Y	8/13 (53.3)	3.73(1.45,9.64)
	-	Vaccine	N	5/37 (14.3)	
Disease[4]	+	Ticks on	Y	3/15 (20.0)	0.62(0.18,2.10)
	-	Site	N	7/19 (36.8)	
Disease	+	Leave Food	Y	4/15 (26.6)	0.96(0.35,2.61)
	-	For Animals	N	10/36 (27.8)	
Disease	+	Vacations	Y	10/31 (32.3)	1.61(0.58,4.49)
	-	Away	N	4/20 (20.0)	
Find Ticks	Y	Ticks On	Y	15/18 (83.3)	0.95(0.74,1.23)
	N	Site Visit	N	21/24 (87.5)	
See Deer Eating	Y	Ticks On	Y	11/18 (61.1)	1.83(0.93,3.62)
	N	Site Visit	N	8/24 (33.3)	

[1]Values are read: "Of the 13 dogs that tested positive to the Lyme disease test, 8 had been vaccinated.

[2] RR= Relative Risk

[3] CI= Confidence Interval

[4] Disease Positives reflect animals that were positive for any of the three diseases tested for.

* Dogs whose owners said they had been vaccinated are 3.73 times more likely of having antibodies to Lyme disease, thus testing positive to the ELISA based LymeCHEK® test which is a significant risk factor.

** None of the relative risk values, with the exception of the first, is statistically significant as the confidence intervals include "1" ($\alpha = 0.05$).

Table 3.7. Relative risks and 95 percent confidence intervals calculated for determining risk factors associated with living in Cumberland County, TN.

Characteristic		Percent no./Tot. no.	RR[1] (95% CI[2])
Lyme Dis.	+	15/23[1] (65.2)	1.36(0.63,2.94)
	-	73/135 (54.1)	
RMSF	+	17/28 (60.7)	0.62(0.55,2.16)*
	-	71/130 (54.6)	
E.canis	+	0/6 (0.0)	----------
	-	88/152 (57.9)	

[1] Results are read "Out of the 23 Dogs testing positive to the Lyme antigen, 15 were from Cumberland County TN.
[2] RR = Relative Risk or additional risk experienced by Cumberland County canines over and above that experienced by Knox County canines.
[3] CI = Confidence Interval
* Since all confidence intervals include (1), none is significant ($\alpha = 0.05$).

CHAPTER IV

DISCUSSION

i - Tick Control

The tick control project concluded its third and final year in the fall of 1996. In the beginning of 1997 the feeders were moved further out into the perimeter of Fairfield Glade (FFG) to the border of Catoosa Wildlife Refuge. Three, new sampling sites were established around the new feeder locations, and the old sampling locations (treated and untreated) were sampled as well to determine the long term effects of ivermectin control on the area.

ii - Serosurveys

Overall, the response to the planned clinics by the dog owners was less than desirable. Owner participation was solicited through the use of both media and "hard advertising" techniques. Local radio announcers mentioned several times both clinics, and mention was also made in the local paper "The Glade Sun". Flyers were posted at least two weeks before the clinics in high traffic areas, such as the post office, local golf clubs, and churches. Newspaper articles, according to the residents, worked the best.

Fairfield Glade participation was higher for the most part than was participation for Lake Tansi. Since the outbreak in 1993 was limited to Fairfield Glade, this is not altogether surprising as response to the human serosurveys performed in 1993 was much the same (Standaert et al. 1995). Weather may have also been a factor in the low owner-turnout in Lake Tansi. The first collection date in the spring of 1996 was very cold, and the second in December of 1996 was accompanied by a snow-storm. Other means of soliciting participation in the Lake Tansi community would be necessary for future studies.

The most samples were obtained with the cooperation of the Humane Society of the Tennessee Valley. A great number of samples can be obtained quickly with the assistance of the shelters, but little, if any, information is available for those dogs pertaining to their health and behavioral histories as many are dogs that have been picked up by animal control. If the dog happened to be turned in by an owner, little information would probably be obtained through such an outlet since owners are often reluctant to answer questions.

The sensitivity, specificity and predicted value of these tests are important factors in interpreting the data. Whereas the "gold standard" for RMSF diagnosis is currently the IFA, the definitive test for CE and Lyme disease are PCR and organism isolation and culture, respectively. These

tests are effective for determining exposure at different stages. Whereas the actual values for sensitivity and specificity for the tests used were not readily accessible, one can only assume some margin of error due to inaccurate testing yielding false positives and negatives.

iii - Conclusions and Recommendations

In the future, additional clinics should be held if valuable information is to be obtained about the usefulness of canines serving as a sentinel population. The organization and planning of these events should be scrutinized to maximize the number of participating individuals. In the initial planning stages of this study, mention was made of potentially holding the clinics during rabies clinics. A modification of this idea might be of use in increasing the sample size. Perhaps a "carrot" such as free heartworm screenings could be offered to entice additional owner participation. Unfortunately, such offerings can be expensive, and would in all likelihood require more manpower.

Veterinarian participation would probably be the best approach to soliciting participation. If a bleeding kit were given to the veterinarians containing explicit information including storage tubes and aliquoting instructions. Unfortunately, while this would be an

efficient means of acquiring samples, the risk of losing the questionnaire information and site visit permission is ever present. This would also require a great deal of work on the veterinarians' part and could possibly be a hindrance to their everyday operations and procedures, and the proper equipment for blood fractioning and storage might not be readily available. Limiting collections to only the veterinarians would also enter bias into the data set as only those dogs who visit veterinarians would be sampled. Consequently, a combination of several blood collection options would be beneficial. Veterinarian participation may also be peaked with the detection of the presence of the disease organism DNA by PCR in owner dogs.

Another outlet of owner solicitation which might be employed is arranging site visits at definite times with the dog-owners when an environmental assessment could be done, blood could be drawn, and owner questionnaires could be completed all in one day. Scheduling could possibly be achieved through the use of pre-postage-paid owner interest postcards which could be placed in strategic areas. These cards would allow the owner to indicate good visit times and would require far less effort on the owner's part. This type of collection strategy would avoid the need for a collection site and would probably reduce the amount of necessary manpower. As we performed 15 homesite visits regularly per day, it would probably be possible to perform

10 site visits per day to collect all categories of information.

A combination of both a reward for allowing a blood sample be taken, veterinarian participation, and house-call visitation will probably be the solution to low owner participation. It will probably take several more years to have a data set large enough to draw conclusions on the usefulness of dog populations as sentinel species for predicting the occurrence of tick transmitted diseases. Increased support also may be obtained through the renewed sampling from the human population that will be carried out by Standaert in the same community as part of a follow up to the 1993 study (Standaert, Pers. Comm.).

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APPENDICES

APPENDIX 1. Owner questionnaire

Cumberland County Tick Disease Survey

Circle one (Fairfield Glade), (Lake Tansi), (Other)

Owner Questionnaire:

Name:_____

Address:_____

_____

Phone:_____

1) If you live in the Fairfield Glade/Lake Tansi area, how many open lots are there between you and your nearest neighbor? _____Your 2nd nearest neighbor?_____

2) What is the size lot on which you live? Check one:

Lot size: (less than ½ acre)____ (½ to 1 acre)____ (1+ to 5 acres)____ (more than 5 acres)____

3) How would you describe the land you live on? (Check one) wooded___, shrubby growth___, pasture or farmland___, grass or lawn___, other_____(please specify)

4) In what type of dwelling do you live? (Check one) single dwelling home___,condominium___, trailer___other___(please specify)_____

5) How far is your home from the nearest woods?_____feet

6) What sorts of animals are commonly seen near your home? (Excluding birds)

_____

7) Do you, or other people in your home, leave food or salt blocks out for deer? **Yes/No**

8) Do you leave food for other animals? **Yes/No**

9) Do you ever see deer eating on your property? **Yes/No**

10) Do you have your lawn professionally cared for in any way? **Yes/No**

If yes, who does the work?_____**may we contact them about what chemicals they use? **Yes/No**

Dog Questions:

1) How many dogs do you have? ____(Please enter information for each dog separately in the table.)

2) Do you have any other pets? **Yes/No** (What?_____)

Dog Table

Name	Sex	Breed	Age	Fixed	Years Owned
	M/F			**Yes/No**	
	M/F			**Yes/No**	
	M/F			**Yes/No**	
	M/F			**Yes/No**	

3) How long has your dog lived at your residence?_____years (If anywhere else during the last 6 years, where and How long)_____

4) Has your dog left your residence for any appreciable length of time in the last year?(e.g. vacations etc.) **Yes/No**

5) Does your dog live outdoors/indoors/indoor-outdoor? (Please circle one.)

6) During a 24 hour period, how long does your dog spend out doors? ½ to 1 hour___,1-2 hours___,2-5 hours___, more than 5 hours___ (Please check one)

7) When outside, is your dog (Please check one) on a

leash___, free running___, or fenced in___

(invisible___/physical___)?

8) Do you feed your dog outside? **Yes/No**

9) Do you walk or exercise your dog in your neighborhood **Yes/No**, and/or do you take your dog elsewhere to walk it. **Yes/No** (If so, where?_____)

10) How far from your house do you walk your dog?_____

11) Have you ever found ticks on your pet? **Yes/No**

If so, check the one that applies. frequently___, occasionally___, infrequently___, rarely___.

12) Does your dog wear a flea/tick collar? **Yes/No**

13) Do you use other treatments for flea and/or tick control? **Yes/No** (If so, what products are you using?

_____)

14) Name of veterinarian._____May we contact them? **Yes/No**

15) Has your dog been vaccinated for Lyme Disease? **Yes/No**

16) Is your dog on Heartworm preventive medication? **Yes/No**

***see attached release form

17) Has your dog ever been diagnosed with Lyme Disease? **Yes/No**

18) Has your dog ever been diagnosed with Rocky Mountain spotted fever? **Yes/No**

19) Has your dog ever been diagnosed with Ehrlichia? **Yes/No**

20) Has your dog had any of the following symptoms during the past six months.

**Circle all that apply.

depression

loss of appetite

weight loss

diarrhea

difficulty breathing

spotted nasal area

lameness

loss of vigor

arthritis

nosebleed

vomiting

spotted mouth

pale to blue mouth and

coughing

stiffness

blood in urine

Veterinarian release form:

I,_____, do hereby give the members of the UT tick research team permission to obtain the medical history of my pet, _____, from my veterinarian,_____.

Print Name: _____

Signature: _____

*** Can tick researchers from the University of Tennessee come to your residence and survey for ticks, or contact you by phone for additional information? **Yes/No**

APPENDIX 2. Home Site Evaluation Form

ID#_____Owner:_____
Lot#_____G.P.S.coordinates_____
Address:_____
_____
Phone:_____Dog's Name:_____

open lots between house and nearest neighbor_____, 2nd nearest neighbor?_____

Size of the lot.< ½ , ½ -1, 1+ to 5, >5 acres.

Site vegetation description: Shrubby growth, wooded, pasture or farmland, grass or lawn.

Type of dwelling._____Distance to woods._____

Rating Scale: 1=1-20%, 2=21-40% etc.
On Site:
Over story percent 1-2-3-4-5 Spp. Hardwood, pine, mixed
Under story percent.1-2-3-4-5 Spp. Pine, blueberry, shrubby growth, grass

Adjacent sites:
Over story Percent and Species:
Front:1-2-3-4-5 Spp. Hw, P, M
Front Right:1-2-3-4-5 Spp. Hw, P, M
Front Left:1-2-3-4-5 Spp. Hw, P, M
Back:1-2-3-4-5 Spp. Hw, P ,M

Under story Percent and Species:
Front:1-2-3-4-5 Spp. P, BB, SG, G
Front Right:1-2-3-4-5 Spp. P, BB, SG, G
Front Left:1-2-3-4-5 Spp. P, BB, SG, G
Back:1-2-3-4-5 Spp. P, BB, SG, G

# drags	Females	Males	Nymphs	Larval Masses
Site				
Front				
Front R.				
Front L.				
Back				

Comments:

APPENDIX 3 - Letter to the owners

Dear Ms. Doe,

Your dog, Fido, was recently tested for Rocky Mountain Spotted Fever, *Ehrlichia chaffeensis*, and Lyme disease. Your dog tested positive to the Rocky Mountain spotted fever test with a titer of 1:320, and negative for both *Ehrlichia chaffeensis* and Lyme disease. The test used for Rocky Mountain spotted fever is an antigen-type indirect antibody assay developed in our diagnostic laboratory. (Substitute Lyme test results and type of test).

There are three explanations possible for the results we obtained on your dog(s). One, is the test was wrong. Occasionally scientific tests of this nature yield an incorrect, or false, positive result. An objective of our project is to develop more definitive tests for these diseases so as to better inform dog owners of their dog's true exposure and infection status, thus eliminating such false results. A second explanation might be your dog(s) at one time came in contact with the disease organism but is currently not infected. Such animals may maintain antibodies (i.e. maintain a positive titer) to the organism as a residual defense mechanism. In the case of Lyme disease, a vaccinated dog may test positively but not be infected. Lastly, your dog might be currently infected with the disease organism. If this is the case, your dog might be showing non-specific signs of illness such as lethargy, loss of apatite, fever, lameness, or bruising of the skin.

We encourage your discussing these findings with your veterinarian. If your dog is currently healthy and is not exhibiting any visible health problems the first two explanations offered above are the most likely. However, if your dog is currently ill, the third explanation might be valid in which case, we recommend you contact your

veterinarian as soon as possible.

In discussing these findings with your vet, they might recommend a re-test to rule out the false positive explanation. Should this be the recommendation, we will gladly provide that service free of charge. In order to do this, we need a vial of serum (at least 1 ml) taken by the dog's veterinarian and sent to the address below:

UTCVM
2407 River Dr.
Pathology Dept. A-239
Knoxville, TN 37996
Attention: Terri Doan

We appreciate your continued support of the study we are conducting in your community. If your veterinarian has any questions regarding the results of these tests they are welcome to contact Dr. John New at the College of Veterinary Medicine, (423)-974-5576. If you have other questions about this project , please feel free to contact one of us at (423) 974-7135.

Thank you,

Reid R. Gerhardt, Ph.D.
Eric Marsland

VITA

Eric John Marsland grew up in Richmond, VA where he graduated from Trinity Episcopal school with a concentration in science. From there he attended The University of the South in Sewanee, TN where he graduated with a B.S. in biology. While there he performed research on the lamin proteins of the nuclear envelope and their role in mitotic division.

In August of 1995, he entered the Department of Entomology and Plant Pathology at the University of Tennessee as a graduate research assistant under the direction of Dr. Reid R. Gerhardt. In December of 1997 he received his Master of Science degree with a major in entomology and a minor in statistics. He hopes to pursue a doctoral degree combining both vector ecology and epidemiology.

The author is a member of the Entomological Society of America, The Tennessee Entomological Society, The Tennessee Academy of Sciences, and Gamma Sigma Delta Agricultural Honor Society.